DIETARY PROTEIN REQUIREMENT OF TWO SIZE CLASSES OF SINGHI, *HETEROPNEUSTES FOSSILIS* (BLOCH)

DISSERTATION

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By

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Certificate

This is to certify that the dissertation entitled **"Dietary protein requirement of two size classes of singhi, *Heteropneustes fossilis* (Bloch)"** has been completed under my supervision by **Ms. Tabassum Qamar Siddiqui.** The work is original and has been pursued by the candidate independently. It embodies some interesting observations contributing to the existing knowledge on the subject. I permit the candidate to submit the work for the award of degree of Master of Philosophy in Zoology of the Aligarh Muslim University, Aligarh, India.

Mukhtar A. Khan

Dr. Mukhtar A. Khan
Reader

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TABASSUM

Tabassum Qamar Siddiqui

DEDICATED TO MY PARENTS

GENERAL INTRODUCTION

INTRODUCTION

During the last fifty years enormous growth of human population has taken place all over the world, especially in the developing countries. Millions of human beings suffer from hunger and malnutrition. Although agricultural production has also increased substantially but per capita availability of food is still low and there is shortage of protein, fat and calories in diet of an average man. Nutritional problem are not peculiar to our country where food is scarce. Protein calorie malnutrition is in fact a serious disease affecting nearly half of the world population. Fish and other forms of sea food can be used to provide balance diet and reduce the protein gap. In the face of nutritional inadequacies resulting from ever increasing population of the developing countries, aquaculture holds a promise to provide a proteinaceous supplement to human diet.

Fish ranks first among the farmed animals in terms of protein yields per unit food intake. The importance of fish is not only undiminished but is on the other hand on the increase for the reason that fish happens to be one of the important sources of protein for the people and has a global market. Fish is food of an excellent nutritional value, providing high quality protein with balanced amino acid profile. It contains low fat than chicken, mutton and pork. Its fat or lipid contains less cholesterol and more polyunsaturated or highly unsaturated fatty acid. Fish as a food is less costly and its use as food will reduce heart disease in human being. Fish also contains vitamin A and D in sufficient quantities than other animal meat products. It is believed that fish eating children could be more intelligent. This is possible because of the more of highly unsaturated fatty acids which helps in the development of brain or cellular membrane synthesis in early part of life. It is easily digestible as compared to beef and poultry. It provides several important byproducts like fish liver oils, fish body oil, fish manure, fish silage, fish glue fish leather, fish flour, biscuits and artificial pearls, etc., and also prevents several nutritional deficiencies. Fish flesh contains all the amino acids and also provides calcium, magnesium, potassium, sodium, iodine, phosphorus and vitamins. The entire need of food for peoples can not be met from land only. The importance of fish as food has resulted in the development of fisheries as an industry in several countries and considerable progress has been achieved in the fisheries science. Fisheries sector plays a vital role in the socio-economic development of the country. In addition to being the fastest growing food production sector of the world, aquaculture activities currently employ about 9 million people (FAO 2000). Apart from generating food production, it stimulates the growth of a number of subsidiary industries for processing and production of various value added fish products so much so that the sector has been recognized as a powerful employment and income generator.

Increased fishing is dwindling our resource and hence there is a need to find an alternative means of increasing fish production. This can be achieved by aquaculture which is an important weapon in the global fight against malnutrition and poverty, particularly in the developing countries (Tacon 2001). Increase in human population along with changing perceptions of healthy food is set to increase the demand for fish. The total fish catch from the world's fishing grounds have levelled off in the last decade with the majority of wild stocks being fully exploited. The need for substantial increase in the world supply of animal protein has generated greater interests in aquaculture of finfish, shrimps and other aquatic organism. Aquaculture production seems to be responding to the increased fish demand and have exclusively increased the world fish production. The food and agriculture organization, in its report on the world situation of fisheries and fish farming, advanced the prediction that the world production of fish, the total consumption and the food demand and consumption per capita would increase during the next three decades. It also indicated that world production of fish from capture fisheries would stagnate, whereas that from fish farming would increase. The average world consumption of fish per person could grow from 16 kg a year in 1997, to 19-20 kg by 2030, raising the total food use of fish to 150-160 million metric tones annually. Hence, there is a need to increase production of fish both fresh water and marine by applying new scientific methods and technologies in aquaculture sector. Out of 20,000 different species of the fish, only 100 species are cultured for commercial purpose.

An important prerequisite to successful aquaculture is the knowledge of the nutritional needs of the species concerned leading to the development of economical feed mixtures. Recent years have therefore seen much interest in this field. The rearing of fish at high stocking levels necessitates a detailed study of their nutritional requirement in order to produce feeds that besides being cost effective are nutritionally adequate for their growth. Hence, the economic success of the controlled production of fish depends mainly on the cost of the feed and particularly on that of protein, since protein is the major and most expensive determinant for growth (Borlongan 1991). Fish generally have higher protein requirement than terrestrial animals (Lovell 1989; Patnaik et al. 2005) and the requirement is influenced by species, age of the animal and water temperature. Fish culture in tropical climate have lower protein requirement (25-35%) than those cultured in moderate climate (30-40%). Fish fry has the higher protein requirement which declines with the growth. Warmwater fishes have a faster specific growth rate than temperate fishes. Results of most protein requirement studies indicate that fish need relatively high (35-55%) protein for their growth (NRC 1993; Tacon & Cowey 1985; Wilson & Halver 1986; Moore et al. 1988; Tibbetts et al. 2000; Lupatsch et al. 2001; Ai et al. 2004; Mayer & Fracalossi 2004; Wanwiza et al. 2005; Sa et al. 2006). Protein requirement are always studied in aquaculture species with the aim of determining the minimum amount required to produce maximum growth. Since protein constitutes in fish culture the single most expensive item in artificial feeds, it is logical to incorporate only that much which is necessary for normal maintenance demand and growth. Any excess is considered wasteful biologically as well as economically. Decrease in protein requirement of fish with increasing size or age has been observed for several warmwater fish species (Halver 1982; Sen et al. 1978). Diet development for particular species therefore requires a precise assessment of its protein needs which determines to a large extent the overall success of its production. The pioneering work of Halver and his colleagues, working on chinook salmon with diet containing casein, gelatin and crystalline amino acid, provided the basic model for subsequent studies on the protein nutrition of a number of the fish species.

Knowledge of the protein requirement is essential for formulation of well-balanced low cost and environment friendly artificial diets because it is the principal diet component for animal growth and is the highest cost consideration in commercial feeds (Lim et al. 1979; Mai et al. 1995; Manomaitis 2001; Thompson et al. 2004; Ozorio et al. 2006, Debnath et al. 2007). It is mainly for this reason that considerable attention has been given in the past to protein nutrition in fish (Wilson 2002; Sales et al. 2003; Luo et al. 2004; Abbas et al. 2005, Kvale et al. 2007).

Aqua-feed section of India has made tremendous development during the last two decades and annual growth rate of 10% in aquaculture is the highest among the other agriculture sectors. Significance of research and human resource development in aqua feed sector has also contributed to high growth rate in aquaculture. At present about 20 million tons of manufactured aqua feeds are being used in aquaculture sector of which major share is being used in shrimp culture. If this growth persist the feed requirement may increase many folds. Hence, more scientific understanding and interventions are required for sustainable aquaculture of our country.

This high dietary protein requirement in fish is generally attributed to preferential use of protein over carbohydrates as a dietary energy source (Cowey et al. 1975). The optimum level of protein in the diet is however, influenced by factors such as the balance of essential amino acids, protein digestibility and protein energy ratio in diet besides temperature of water, salinity, stage of growth of the fish and species and availability of natural food (Mertz 1969; Cowey & Luquet 1983; De Silva & Pereira 1985).

Dietary protein requirements have been determined for many fish species like chinook salmon, *Oncorhynchus tschawytscha* 40 (De Long et al. 1958), sockeye salmon, *O. keta* 45 (Halver et al. 1964), common carp, *Cyprinus carpio* 31–38 (Ogino & Saito 1970), Japanese eel, *Anguilla japonica* 44.5 (Nose and Arai 1972), plaice, *Pleuronectes platessa* 50 (Cowey et al. 1972), gilthead bream, *Chrysophrys aurata* 40 (Sabaut & Luquet 1973), coho salmon, *O kisutch* 40 (Zeitoun et al. 1974), rainbow trout, *O. mykiss*

40 (Satia 1974), yellowtail, *Seriola quinquiradiata* 55 (Takeda et al.1975), channel catfish, *Ictalurus punctatus* 32–36 (Garling & Wilson 1976), red sea bream, *Pagrus major* 55 (Yone 1976), Atlantic salmon, *Salmo salar* 45 (Lall & Bishop 1977), grass carp, *Ctenopharyngodon idella* 41–43 (Dabrowski 1977), estuary grouper, *Epinephelus coioides* 40–50 (Teng et al. 1978), common carp, *Cyprinus carpio* 31-38 (Takeuchi et al. 1979), Zillii's tilapia, *Tilapia zilli* 35 (Mazid et al. 1979), milkfish, *Chanos chanos* 40 (Lim et al.1979), Zillii's tilapia, *Tilapia zilli* 50 (Kanazawa et al.1980), small mouth bass, *M. dolomieu* 45 (Anderson et al. 1981), largemouth bass, *Micropterus salmoides* 40 (Anderson et al. 1981), blue tilapia, *Oreochromis aureus* 34 (Winfree & Stickney 1981), snakehead, *Channa sps.* 52 (Wee & Tacon 1982), mozambique tilapia, *O. mozambichus* 40 (Jauncey 1982), tilapia nilotica fry, *O. niloticus* 35 (Santiago et al. 1982), striped bass, *Morone saxatilis* 47 (Millikin 1983), Nile tilapia, *O. niloticus* 30 (Wang et al.1985), young tilapia, *Oreochromis. sps.* 28 (De Silva et al. 1989), walking catfish, *Clarias batrachus* 40 (Khan & Jafri 1990), Malaysian freshwater catfish, *Mystus nemurus* 42 (Khan et al. 1993), brown trout, *Salmo trutta* 53 (Arzel et al. 1995), hybrid *Clarias* catfish, *C. macrocephalus x C. gariepinus* 40 (Jantrarotai et al. 1996), Eurasian perch, *Perca fluviatilis* 43-56.1 (Fiogbe et al. 1996), American eel, *Anguilla rostrata* 47 (Tibbetts et al. 2000), bagrid catfish, *Mystus nemurus* 44 (Ng et. al. 2001), juvenile haddock, *Melanogrammus aeglefinus* 49.9 (Kim & Lall 2001), juvenile ayu, *Plecoglossus altivelis* 38 (Lee et al. 2002), singhi, *H. fossilis* 40 (Deepak & Garg 2003), juvenile spinibarbus, *Spinibarbus hollandi* 32.7 (Yang et al. 2003), South African abalone, *Haliotis midae* 35.9 (Sales et al. 2003), Indian major carp 40 (Kalla et al. 2004), Mahseer, *Tor putitora* 45-50 (Islam and Tanaka 2004), red snapper, *Lutjanus argentimaculatus* 44

(Catacutan et al. 2001), juvenile grouper, *Epinephelus coioides* 48 (Luo et al. 2004), Australian redclaw crayfish, *Cerax quadricarinatus* 32 (Jacinto et al. 2005), bagrid catfish, *Pseudobagrus fulvidraco* 42 (Kim & Lee 2005), juvenile turbot, *Scophthalmus maximum* 55 (Cho et al. 2005), juvenile haddock, *Melanogrammus aeglefinus* 54.6 (Tibbetts et al. 2005), white sea bream, *Diplodus sargus* 38-42 (Sa et al. 2006) per cent protein.

As far as aquaculture of Indian cultivable fish species is concerned, a number of carp species having good growth and market demand are being cultured. These species contribute to aquaculture production substantially. However, culture of air-breathing fish has also gained attention because of the growing demand for fish. The optimum level and quality of dietary protein to include in commercial catfish diets are dependent on several factors, including the balance between energy and protein in the diet, the amino acid composition of the diet, and feeding rate. Protein is needed to promote growth, while less expensive feed ingredients like corn and wheat are sufficient for providing energy. Catfish diets should be balanced to ensure that adequate levels of protein and the less-expensive energy sources are supplied in proper proportions to minimize the use of protein for energy and to maximize protein deposition. Increasing protein level in diet can lead to improved fish production especially for carnivorous fish.

Information on the basic nutritional requirement and feeding of catfishes which form a major group of commercially important fish species after carp is needed in view of recent emphasis on catfish culture in the country. *Heteropneustes fossilis,* the fish under study, is one of the most easily cultivable indigenous air-breathing catfishes and is of high nutritive value. It is highly esteemed as food fish fetching a high price. It takes at least 1 year to reach marketable size.

Although dietary protein requirements of other cultivable fish species including carps have been worked out (Sen et al. 1978; Renukaradhya and Varghese 1986; Singh & Bhanot 1988; Shim et al. 1989; Khan & Jafri 1991; Webster et al. 1994; Jacinto et al. 2003; Khan et al. 2004; Lee and Sang 2005; Usman et al. 2005; Debnath et al. 2007), little information is available on the protein requirement of singhi, *H. fossilis*. Studies leading to the knowledge of optimum dietary protein requirement for this species will be a useful step in developing nutritionally adequate quality protein diets for the intensive culture of this fish. The present study was, therefore, undertaken to generate data on dietary protein requirement of two size classes of *H. fossilis* and the findings are presented in the form of this dissertation.

GENERAL METHODOLOGY

Rearing of fish

GENERAL METHODOLOGY

Source of fish stock and their acclimatization

Induced bred *Heteropneustes fossilis* were procured from a local fish hatchery. These were transported to the wet laboratory in oxygen filled polythene bags, given the prophylactic dip in $KMnO_4$ solution (1:3000), and stocked in circular aluminium plastic lining (Plastic Crafts Corpn., Mumbai, India, 4ft x 3ft x 3ft) fish tanks (water volume 600L) for a fortnight. During this period, the fish were fed to satiation a mixture of soybean, mustard oil cake, rice bran and wheat bran in the form of moist cake twice a day at 0900 and 1630 hours. These were then acclimatized for one week on casein-gelatin based (40% CP) H-440 diet (Halver 2002) near to satiation.

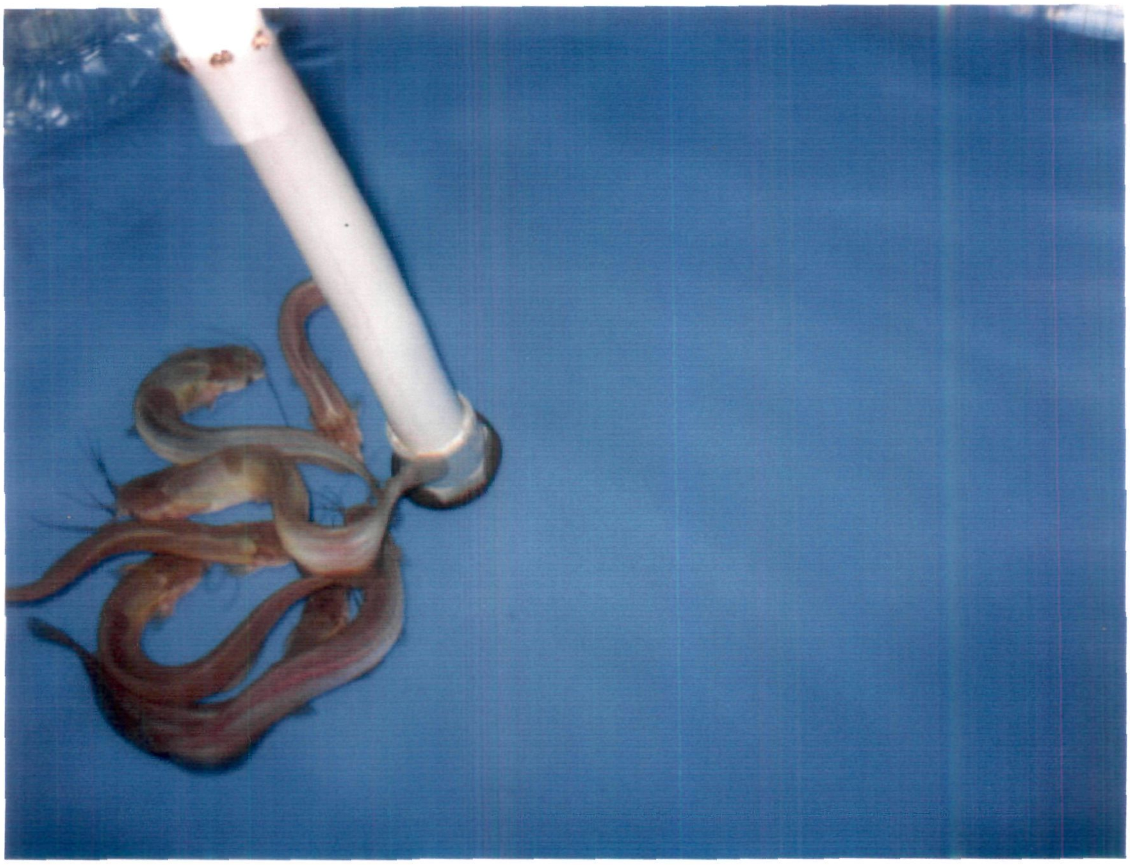
Preparation of experimental diets

For studying the dietary protein requirement of *Heteropneustes fossilis,* test diets were formulated with graded levels of proteins. The dietary range necessary to quantify the protein requirement was adjusted on the basis of information available on other catfish species. Calculated quantities of dry ingredients were thoroughly stirred in a volume of hot water (80 °C) in a steel bowl attached to a Hobart electric mixer (Hobart Corporation, Troy, OH, USA). Gelatin powder was dissolved separately in a volume of water with constant heating and stirring and then transferred to the above mixture. Other dry ingredients and oil premix, except carboxymethyl cellulose, were added to the lukewarm bowl one by one with constant mixing at 40 °C temperature. Carboxymethyl cellulose was added last and the speed of the blender was gradually increased as the diet started to

Flow-through system used for feeding trial harden. The final diet, with the consistency of bread dough, was poured into a teflon-coated pan and placed into refrigerator to gel. The prepared diet was in the form of moist cake (50% dry matter) from which cubes were cut and stored at -20 ^{0}C in sealed polythene bags until used.

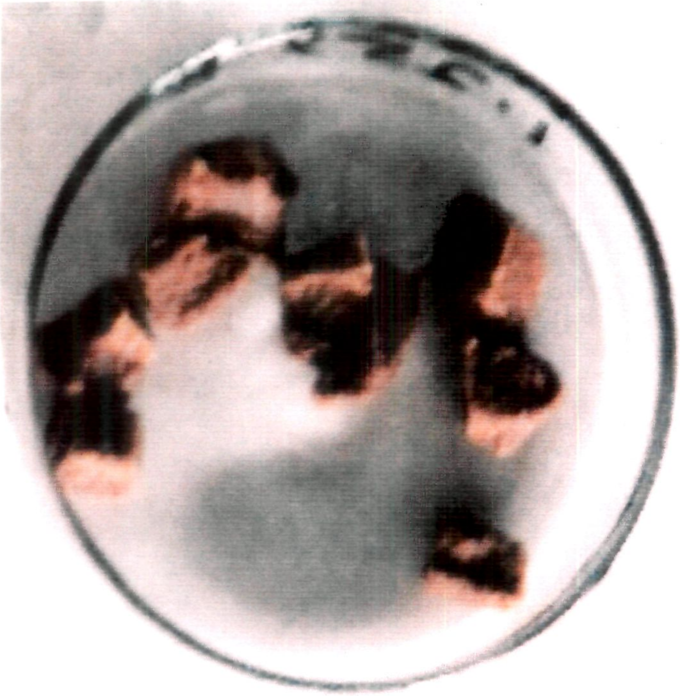
Feeding trial

Fish of the desired size and number were sorted out from the acclimatized fish lots maintained in the wet laboratory. These were stocked in triplicate groups in 70L high density polyvinyl circular troughs (water volume 55L) fitted with continuous water flow-through system. The water exchange rate in each trough was maintained at 1.0-1.5 L/min. After carefully observing the feeding behaviour of the fish and feed intake, they were fed ad lib six days a week twice a day at 0900 and 1630 h. The feeding trials lasted for 8 weeks. Initial and weekly body weights were recorded on a top loading balance (Precisa 120A; PAG, Oerlikon, AG, Zurich, Switzerland). Troughs were siphoned off to remove faecal matter before feeding daily. Accumulation of the diet at the bottom of the trough was avoided. Uneaten feed, if any, was siphoned off immediately, dried in a hot air oven and reweighed to measure the amount of feed consumed. No feed was given on the day of weekly measurement. Troughs were scrubbed and disinfected thoroughly with water and $KMnO_4$ solution on the day of weekly measurement. Mortality, if any, was recorded. At the end of the experimental trial, desired number of fish were randomly sacrificed and kept in freezer (-20 ^{0}C) for the assessment of whole body composition.



Fish being acclimatized on experimental diet

Fish being acclimatized on experimental diet



Pelican equipments Socs Plus used for the estimation of fat

Proximate analyses

Assessment of proximate composition of ingredients, diets and carcass was made using standard techniques (AOAC 1995). All chemical analyses were based on triplicate samples.

Moisture

A known quantity of sample was taken in a pre-weighed crucible and placed in a hot air oven at 105±1 ^{0}C for 24 hours. After complete drying, the sample was cooled at room temperature in a desiccator and was reweighed. The loss in weight gave an index of water from which its percentage was calculated.

Ash

A known quantity of dried powdered sample (2-5g) was taken in pre-weighed silica crucible and incinerated in a muffle furnace (600 ^{0}C) for 2-4 hours or till the sample became carbon-free and completely white. The crucible was cooled in a desiccator and reweighed to estimate the quantity of ash. The result was expressed as percentage on dry weight basis.

Fat

Crude fat was estimated using Socs Plus SCS 4, Pelican equipments, Chennai, India, by continuous soxhlet extraction technique using petroleum ether (40-60 ^{0}C B.P.) as solvent. Finely powered and dried sample (2-4 g) was placed in a thermocol fat extraction thimble and placed in the soxhlet apparatus. A clean, dry soxhlet receiver flask was weighed and

Kjeltec Tecator TM^{2300} used for the estimation of proteins in the sample

fitted to the soxhlet assembly on a boiling water bath for extraction which was continued for 2-3 hours. After extraction the flask was removed and kept in hot air oven (100 ^{0}C) to evaporate the traces of solvent. It was then transferred to a desiccator, cooled and reweighed. The difference between the weight of the flask before and after gave the quantity of crude fat extracted from the unknown amount of the sample. The result was expressed as percentage on dry weight basis.

Crude protein

The estimation was done using Kjeltec Tecator^{2300}, Foss, Hoeganaes, Sweden. A known quantity of sample was taken in Kjeltec digestion tubes. To this, 0.8g of copper sulphate, 7.0g potassium sulphate and 12 ml. of concentrated sulphuric acid was added. The content was digested in the digester of the instrument. The process of digestion continued for 30 minutes. Now the digested sample was cooled at room temperature and titrated automatically in distillation unit of the instrument. The level of protein displayed on the screen was noted down.

Gross energy

Gross energy was determined on a ballistic bomb calorimeter (Gallenkamp, Loughbrough, England). Prior to estimate, a known quantity of dried powdered sample (0.5-1.0g) was taken in metallic crucible and compacted carefully to increase the rate of combustion at 25 lb oxygen pressure. The heat generated upon combustion was read on the modulated galvanometer scale, and converted to energy equivalent, worked out earlier using the thermo chemical grade benzoic acid (6.32 kcal g^{-1}) as a standard. The gross energy was expressed as kcal g^{-1}. Energy of ingredients used in the test diet was calculated as 5.52, 4.83, 3.83 and 9 kcal g^{-1} for casein, gelatin, dextrin and fat, respectively as estimated on Gallenkamp ballistic bomb calorimeter.

Assessment of growth and conversion efficiencies

Calculations of the growth parameters were made according to the following formulae (Wee and Tacon, 1982; Tabachek, 1986; Hardy, 1989 and Gunasekera et al. 2000, Abidi and Khan, 2007):

$$\text{Gain in live weight (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\log e W_2 - \log e W_1}{D} \times 100$$

W_2 = Final weight of fish

W_1 = Initial weight of fish

D = Duration of the feeding trial (days)

$$\text{Feed conversion ratio} = \frac{\textit{Dry weight of feed consumed}}{\textit{Wet weight gain}}$$

$$\text{Protein efficiency ratio} = \frac{\textit{Wet weight gain}}{\textit{Protein consumed (dry weight basis)}}$$

$$\text{Body protein deposition} = \frac{(BW_f \times BCP_f) - (BW_i \times BCP_i)}{TF \times CP} \text{x100}$$

BW_f & BWi = mean final and initial body weight

BCP_f & BCPi = mean final and initial percentage of body protein

TF = Total amount of diet consumed

CP = Percentage of crude protein in diet

Statistical analysis

Responses of *Heteropneustes fossilis* fed graded levels of protein were measured by live weight gain per cent, feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR) and by analyzing the whole body composition. These response variables were subjected to one-way analysis of variance (Snedecor & Cochran 1968; Sokal & Rohlf 1981). To determine significant differences ($P<0.05$) among the treatment means, Duncan's Multiple Range Test (Duncan 1955) was employed. Second-degree polynomial regression analysis (Zeitoun et al. 1976) was used to find out the break-point in the growth curve and was employed for live weight gain per cent, FCR, PER, SGR and BPD, to predict more accurate response to the dietary intake. The break points obtained represented the optimum requirement for protein.

Table 1. Composition of mineral mixture*

Minerals	**g 100g^{-1}**
Calcium biphosphate	13.57
Calcium lactate	32.69
Ferric citrate	02.97
Magnesium sulphate	13.20
Potassium phosphate (Dibasic)	23.98
Sodium biphosphate	08.72
Sodium chloride	04.35
Aluminium chloride.6H2O	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Magnus sulphate.H2O	0.080
Cobalt chlcride.6H2O	0.100
Zinc sulphate	0.300

*Halver 2002

Table 2. Composition of vitamin mixture*

Vitamins	g $100g^{-1}$
Alpha cellulose	2.000
Choline chloride	0.500
Inositol	0.200
Ascorbic acid	0.100
Niacin	0.075
Calcium pantothenate	0.050
Riboflavin	0.020
Menadione	0.004
Pyridoxine HCl	0.005
Thiamin HCl	0.005
Folic acid	0.0015
Biotin	0.0005
Alpha tocopherol acetate**	0.040
Vitamin B_{12}***	0.00001(0.5 ml)

*Halver 2002
**Incorporated with oil
***(10mg/500ml H_2O)

CHAPTER 1

CHAPTER 1

DIETARY PROTEIN REQUIREMENT OF FINGERLING *HETEROPNEUSTES FOSSILIS* (BLOCH)

INTRODUCTION

The economic success of the controlled production of fish depends mainly on the cost of the feed and particularly on that of protein (Borlongan 1991) therefore, protein requirement studies on fish are the most important aspect of aquaculture and are one of the first and foremost necessary information for successful culture (Debnath et al. 2007). The major function of the dietary protein is to supply amino acids required by fish for growth and reproduction. Assessment of the quantitative requirement of dietary protein of fish under culture conditions enables the formulation of well-balanced and cost-effective feeds. Protein requirement are always studied in aquaculture species with the aim of determining the minimum amount required to produce maximum growth. Since protein constitutes in fish culture the single most expensive item in artificial feeds, it is logical to incorporate only that much which is necessary for normal maintenance demand and growth. Any excess is considered wasteful biologically as well as economically.

Culture of the air-breathing fish has gained attention because of the growing demand for fish protein. The siluroid, *Heteropneustes fossilis* is an important tropical fresh water food fish. In recent year the farming of this species has gained considerable importance in India. Although for the economical production of this species conventional feeding strategies such as feeding combination of trash fish, oil cake, etc , are generally adopted, very little information is currently available on the nutrient requirement of *H.*

fossilis. Decrease in protein requirement of fish with increasing size or age has been observed for several warmwater fish species. Although, in the past protein requirement has been worked out for a number of fish species including juvenile sunshine bass (Brown et al. 1992), red sea bream, *Pagrus major* (Yone 1976), milkfish, *Chanos chanos* (Lim et al. 1979), smallmouth bass, *Micropterus dolomieu* (Anderson et. al. 1981), mossambique tilapia, *Oreochromis mozambichus* (Jauncey 1982), Nile tilapia, *O. niloticus* (Wang et al. 1985), Nile tilapia, *O. niloticus* (Kaushik et al. 1995), juvenile dentex, *Dentex dentex* (Tibaldi et al. 1996), gilthead sea bream, *Sparus aurata* (Lupatsch et al. 1998), African catfish, *Clarias gariepinus* (Ali & Jauncey 2005), milkfish, *Chanos chanos* (Jana et al. 2006), juvenile blackspot sea bream, *Pagellus bogaraveo* (Silva et al. 2006) but information on the protein requirement of fingerling *H. fossilis* is lacking. The present study was, therefore, conducted to evaluate the protein requirement of fingerling *H. fossils*. The information would be useful in developing protein balanced diet for the culture of this fish species.

Materials and methods

Preparation of diet

Six iscaloric (17.37 kJ g^{-1}) diets containing graded levels of protein (25, 30, 35, 40, 45 and 50%) were formulated (Table 1). The dietary range necessary for assessing the protein requirement was adjusted on the basis of information available on other catfishes. Diets were made isocaloric by adjusting the dextrin.

Method of preparation of experimental diets is the same as discussed under general methodology section (Page-9-10).

Feeding trial

Source of the fish, their acclimation and details of the general experimental design has already been discussed under the general methodology section (Page 10).

Fingerling *H. fossilis* (5.01±0.05 cm; 3.81±0.02g) were stocked randomly in triplicate groups in 70-L circular polyvinyl troughs (water volume 55L) fitted with a continuous flow-through system at the rate of 20 fish per troughs for each dietary treatment levels. The fish were fed experimental diets in the form of moist cake to apparent satiation divided over two feeding schedules at 0900 and 1630h. No feed was offered to the fish on the day they were weighed. The feeding trials lasted for 8 weeks. Initial and weekly body weights were recorded on a top loading balance (Precisa 120A; PAG, Oerlikon, AG, Zurich, Switzerland). Troughs were siphoned off to remove faecal matter before feeding daily. Uneaten feed if any, was siphoned off immediately, dried in a hot air oven and reweighed to measure the amount of feed consumed. Water quality parameters were measured daily according to standard methodology (APHA 1992). The average water temperature, dissolved oxygen, free carbon dioxide, pH and total alkalinity based on daily measurements were 27.5-27.9 °C, 68-7.5 mg L^{-1}, 5.5-108 mg L^{-1}, 7.5-7.8 and 65.7-80.9 mg L^{-1}, respectively.

Chemical analyses

Proximate composition of casein, gelatin, experimental diets, and initial and final carcass was analyzed using standard methods (AOAC 1995) for dry matter (oven drying at 105± 1 °C for 22 h.), crude protein (N-Kjeldahl X 6.25 using Kjeltec Tecator™ Technology 2300, Sweden), crude fat (solvent extraction with petroleum ether B.P 40-60 °C using

Socs Plus SCS 4, Pelican equipments, Chennai, India, for 2-3 h.) and ash (oven incineration at 650 °C for 4-6 h). Gross energy content was determined on a Gallenkamp ballistic bomb calorimeter-CBB 330 010L (Gallenkamp, Loughbrough, U K). Six subsamples of a pooled sample of 40 fishes were analyzed for initial body composition. At the end of the experiment, all 20 fishes from each replicate of dietary treatments were pooled separately and analyzed for final body composition.

Statistical analysis

All growth data were statistically processed for the analysis of variance (Snedecor & Cochran 1968; Sokal & Rohlf 1981). Differences among treatment means were determined by Duncan's Multiple Range Test at a $P<0.05$ level of significance (Duncan 1955). To predict more accurate response to the dietary intake, the break-point for optimum dietary protein requirement for fingerling *H. fossilis* was estimated using second-degree polynomial regression analysis ($Y=aX^2+bX+c$) as described by Zeitoun *et al.* (1976). All the statistical analysis was done by Matlab (version 6.5) and SPSS (version 11.3).

Results

Growth performance of the fingerling *H. fossilis* fed diet containing graded levels of protein for 8 weeks are shown in Table 2. Live weight percent, FCR, PER and SGR were significantly ($P<0.05$) affected by dietary protein levels and improved with inclusion of protein in the diet upto 45%. Maximum live weight gain percent (213%), best FCR (1.35), highest PER (1.65) and SGR (1.99) were observed in fish fed diet containing 45%

protein (Diet V). Poor growth and efficiency of feed utilization were evident below this level of protein inclusion. Poorest FCR (3.22), lowest PER (1.24), SGR (1.25) and BPD (11.54) were observed for fish fed diet containing 25% protein (Diet I). No mortality was observed in fish fed the experimental diets during the length of the experiment.

On subjecting the live weight gain data to quadratic regression analysis (Zeitoun *et al.* 1976), a break-point was evident at 43.26% protein of the diet. The relationship was described by the following equations:

$$Y=-0.1217X^2+1.353X-163.86 \ (r=0.982)$$

The FCR of fingerling *H. fossils* protein differed significantly from the other levels of dietary protein inclusion. The FCR (Y) to dietary protein level (X) relationship was estimated by the following second-degree polynomial regression equation:

$$Y=0.0035X^2-0.3387X+9.531 \ (=0.999)$$

Based on the above equation, the estimated FCR occurred at approximately 46.38% protein of the diet.

The PER of fingerling *H. fossilis* fed 45% protein diet differed significantly from the other levels of dietary protein inclusion. The PER (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=-0.0011X^2+0.\ 970X-0.533 \ (r=0.919)$$

Based on the above equation, the estimated PER occurred at a dietary protein level of approximately 43.26% of the diet.

Also, the SGR of fingerling *H. fossilis* fed 45% protein diet differed significantly from the other levels of dietary protein inclusion. The SGR (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=-0.0097X^2+1.0314X-0.734 \ (r=0.996)$$

Based on the above equation, the estimated SGR occurred at a dietary protein level of approximately 46.51% of the diet.

Similarly, the BPD of fingerling *H. fossilis* fed 45% protein diet differed significantly from the other levels of dietary protein inclusion. The BPD (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=-0.0552X^2+0.4910X-77.696 \ (r=0.976)$$

Based on the above equation, the estimated BPD occurred at a dietary protein level of approximately 44.45% of the diet.

On the basis of the above polynomial equations, the maximum live weight gain percent, best FCR, PER, SGR and highest BPD occurred at 43.3, 46, 43, 46.5 and 44.4% protein in the diet, respectively.

Body composition of fingerling *H. fossilis* fingerling was significantly ($P<0.05$) affected by the protein levels of the diets (Table 3). Maximum body protein was recorded in fish fed diet containing 45% protein (Diet V). Body protein was found to increase significantly with the increase in dietary protein upto 45% however, a significant fall in body protein was recorded in fish fed diet containing 50% (Diet VI) protein. Body protein deposition (BPD) values are also influenced by the levels of dietary protein and it increased significantly ($p<0.05$) in fish fed diet up to 45% protein (Diet V). The pattern of BPD was similar to that of body protein. Body moisture content tended to increase with increasing dietary protein levels Body fat increased in fish fed diet containing 25% (Diet I), 30% Diet II and 50% (Diet VI) protein. However, no significant differences in body fat were evident in fish fed diets containing 35% (Diet III), 40% (Diet IV) and 50% (Diet VI) protein and the fat content the fish fed 45% of the dietary protein differed significantly from the other levels of dietary protein inclusion. However, no clear trend for body ash content was observed and it remained almost constant among the groups.

Discussion

Aquaculture has been considered as answer to the shortfall from the global capture fisheries production (Sheeno and Sahu 2006). Commercial culture of a fish depends on the cost-effective diets and the major constraints in developing cost-effective diet are the lack of information on the macronutrient requirements of the candidate species especially in terms of proteins. Dietary protein is one of the major components of fish feeds (NRC 1993) that influences the growth and excretion of nitrogenous wastes in aquaculture. An increase in body protein is expressed as growth. Because protein is the most expensive component of a formulated diet, determination of the protein requirement of the target species for culture should be known precisely for the economical benefits of aquaculture production.

The optimum dietary protein level which resulted in maximum live weight gain percent, best FCR, PER and SGR was found to be at 45% protein in the diet. The FCR improve with each increase in the dietary protein content upto 45% protein thereafter, it decreased at higher level of dietary protein inclusion (Diet VI). A slight growth depression as a result of higher level of inclusion of protein in Diet VI (50%) was noted. A growth depression as a result of feeding higher levels of protein in the diet has also been reported for *Chanos chanos* (Lim et al. 1979) and for other mugilid fish species (Papaparaskeva & Alexis 1986; Kalla et al. 2003). The depression in growth may be attributed to the inhibitory effects of high dietary protein on the activity of the digestive enzymes (Jana et al. 2006). Protein utilization of fish for protein deposition decreases with increasing dietary protein levels, probably because more dietary protein is used as energy (Cho & Kaushik 1985). This was clearly observed during the present study where at maximum level of dietary protein inclusion, a decrease in the body protein deposition value was noted. Fish receiving 45% protein in the diet reflected a maximum gain in weight (213%). Highest PER (1.65), SGR (1.99), BPD (34.3) and best FCR (1.35) were evident in fish receiving the dietary protein at the level of 45% of the diet however, a slight reduction in weight was noted for the groups fed 50% protein in the diet. The inferior growth performance of fish fed Diet I, II and III is attributed to low levels of the dietary protein to meet the optimum requirement by the fish.

Based on the second-degree polynomial regression analyses of growth data the optimum protein requirement for fingerling *H. fossilis* is in the range of 43.3-46.5% of the diet. The value obtained during the present study is higher than the values reported for young grey mullet, *Mugil capito* 24, (Papaparaskeva et al. 1986), Nile tilapia, *Oreochromis niloticus* 25 (El-Saidy & Gaber 2005), juvenile golden shiners, *Notemigonus crysoleucas* 29 and goldfish, *Carassius auratus* 32 (Lochmann & Philips), walking catfish, *Clarias batrachus* 30 (Chaupoehuk 1987), Nile tilapia, *Oreochromis niloticus* 30 (Siddiqui et al. 1988), shingi, *Heteropneustes fossilis* 27.73-35.43% (Akand et al. 1989), big head carp, *Aristichthys nobilis* 30 (Santiago & Reyes 1991), juvenile silver perch, *Bidynus bidynus* 31 (Yang et al. 2002), South African abalone, *Haliotis midae* 35.87 (Sales et al. 2003), African catfish, *C. gariepinus* 43 (Ali & Jauncey 2005), lower than the requirement for juvenile olive flounder, *Paralichthys olivaceus* 46.4-51.2 (Kim et al. 2002), brown trout, *Salmo trutta* 57 (Arzel et al 1995), African catfish, *Clarias gariepinus* 40 (Degani et al. 1989), juvenile masu salmon, *Oncorhynchus masu* 40 (Lee & Kim 2001), juvenile blackspot sea bream, *Pagellus bogaraveo* 40 (Silva et al. 2006), Malaysian catfish, *Mystus nemurus* 42 (Khan et al. 1993), bagrid catfish *M. nemurus* 44 (Ng et al 2001), grouper, *Epinephelus malabaricus* 44 (Shiau & Lan 1996) and slightly lower than the requirement reported for American eel, *Anguilla rostrata* 47 (Tibbetts et al. 2000). The greater requirement may be due to the fact that protein requirement of the fish decreases with age and since the fish was in the fingerling stage therefore, the optimum requirement level was high during the present study. It could be concluded from the present study that the diets for fingerling *H. fossilis* should contain 43-46.5% protein for optimum growth and feed utilization

The data generated during the present study on optimum dietary protein requirement for maximum growth of fingerling *H. fossilis* would be useful in formulating protein balanced diet.

Summary

An 8 week feeding trial was conducted to assess the dietary protein requirement of fingerling *H. fossilis* (5.01±0.05 cm; 3.81±0.02 g). Casein-gelatin based isocaloric test diets (17.37 kJ g^{-1} GE) containing graded levels of protein (25, 30, 35, 40, 45 and 50% of the diet), in gradations of 5% were prepared and fed to triplicate groups of fingerling to apparent satiation divided over two feedings at 0900 and 1630 h. Live weight gain per cent (213%), SGR% (1.99), PER (1.65) and BPD (34.29) were significantly higher in fish fed diet containing 45% protein in the diet. Best FCR (1.35) was also recorded at this level. Second-degree polynomial regression analysis of the live weight gain, FCR, PER, SGR and BPD data indicated the dietary protein requirement of fingerling *H. fossilis* to be at 43.3, 46, 43, 46.5 and 44.4% of the protein, respectively. Body composition data also supported the above requirement level. Maximum body protein content and minimum moisture were recorded at this level of protein in the diet. An insignificant ($P>0.05$) and almost constant trend was noted for ash values. Based on the quadratic regression analysis data dietary protein requirement of fingerling *H. fossilis* is estimated to be in the range of 43.3-46% of the protein.

Table 1 Composition of experimental diets used for quantifying the dietary protein requirement of fingerling *H. fossilis.*

Diets						
Ingredients ($g\ 100g^{-1}$, dry diet)	**I**	**II**	**III**	**IV**	**V**	**VI**
Casein[1]	25	30	35	40	45	50
Gelatin[2]	6.25	7.50	8.75	10.0	11.25	12.5
Dextrin	49.74	40.96	32.18	23.39	14.61	5.83
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[4]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[4,5]	3.0	3.0	3.0	3.0	3.0	3.0
α- Cellulose	-	2.53	5.07	7.6	10.14	12.67
Carboxymethyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Total	99.99	99.99	100	99.99	100	100
Calculated Crude Protein	25.0	30.0	35.0	40.0	45.0	50.0
Analyzed Crude Protein	24.93	29.19	34.45	39.65	44.15	49.03
Gross energy[6] ($kJ\ g^{-1}$, dry diet)	17.37	17.37	17.37	17.37	17.37	17.37

[1]Crude Protein (80%), Loba Chemie, India; [2]Crude Protein (97%), Loba Chemie, India.; [3]Loba Chemie, India.; [4]Halver (2002); [4,5]1g Vitamin mix +2g œ-cellulose; [6]Calculated on the basis of fuel values 23.08, 20.199, 16.02 and 37.64 kJ for casein, gelatin, dextrin, and fat, respectively, as estimated on Gallenkamp ballistic bomb calorimeter.

Table 2 Growth and conversion efficiency of fingerling *H. fossilis*

	Dietary protein levels					
	25	**30**	**35**	**40**	**45**	**50**
Average initial weight (g)[1]	3.8 ± 0.01^{c}	3.88 ± 0.01^{c}	4.40 ± 0.02^{a}	3.92 ± 0.03^{b}	4.84 ± 0.02^{a}	4.60 ± 0.01^{a}
Average final weight (g)[1]	7.68 ± 0.02^{e}	8.80 ± 0.02^{d}	11.40 ± 0.02^{c}	10.92 ± 0.03^{c}	15.16 ± 0.02^{a}	13.88 ± 0.02^{b}
Live weight gain [1,2]	101.97 ± 6.6^{f}	128.2 ± 5.9^{e}	157.9 ± 3.9^{d}	178.1 ± 7.4^{c}	213.2 ± 10.7^{a}	201.8 ± 9.1^{b}
Food conversion ratio[1,2,3]	3.23 ± 0.03^{a}	2.56 ± 0.04^{b}	1.96 ± 0.01^{c}	1.59 ± 0.004^{d}	1.35 ± 0.01^{f}	1.37 ± 0.01^{e}
Protein efficiency ratio[1,2,4]	1.24 ± 0.01^{f}	1.30 ± 0.01^{e}	1.46 ± 0.01^{d}	1.57 ± 0.01^{b}	1.65 ± 0.01^{a}	1.54 ± 0.01^{c}
Specific growth rate (%)[1,2,5]	1.25 ± 0.08^{f}	1.47 ± 0.03^{e}	1.69 ± 0.05^{d}	1.83 ± 0.06^{c}	1.99 ± 0.01^{a}	1.97 ± 0.07^{b}
Percentage survival	100	100	100	100	100	100

[1] Mean values of 3 replicates ± SEM; [2] Mean values sharing the same superscripts are insignificantly different ($P>0.05$).
[2]Live weight gain= Final body weight-Initial body weight/Initial body weight x 100
[3]FCR= Dry feed fed/Weight gain
[4]PER= Wet weight gain/Protein fed
[5]SGR%= ln Final body weight-ln Initial body weight/Number of days x 100

Table 3 Body composition of fingerling *H. fossilis*

		Dietary protein levels (g 100^{-1})					
		25	**30**	**35**	**40**	**45**	**50**
Moisture	81.72± 0.04	79.12±0.11^{c}	78.97± 0.01^{b}	78.21±0.01^{b}	78.21± 0.03^{b}	77.38± 0.30^{d}	80.31±0.13^{a}
Protein	11.79± 0.10	13.79± 0.10^{e}	14.65± 0.15^{d}	14.86± 0.14^{c}	15.87± 0.15^{b}	16.25± 0.01^{a}	15.87± 0.10^{b}
Fat	5.41± 0.01	6.33± 0.06^{c}	6.91± 0.03^{d}	7.33± 0.06^{c}	7.98± 0.05^{c}	8.51± 0.04^{b}	8.93± 0.06^{a}
Ash	2.90±0.03	2.35± 0.10^{a}	2.26± 0.04^{b}	2.40± 0.02^{a}	2.37± 0.01^{a}	2.22± 0.02^{b}	2.14± 0.03^{c}
Body protein deposition	-	11.54± 0.05^{f}	19.05± 0.15^{e}	24.67± 0.03^{d}	30.80± 0.12^{b}	34.29± 0.06^{a}	28.05± 0.07^{c}

[1] Mean values of 3 replicates ± SEM; [2] Mean values sharing the same superscripts are insignificantly different (P>0.05).

Fig. 1 Second-degree polynomial regression analysis of live weight gain to dietary protein level

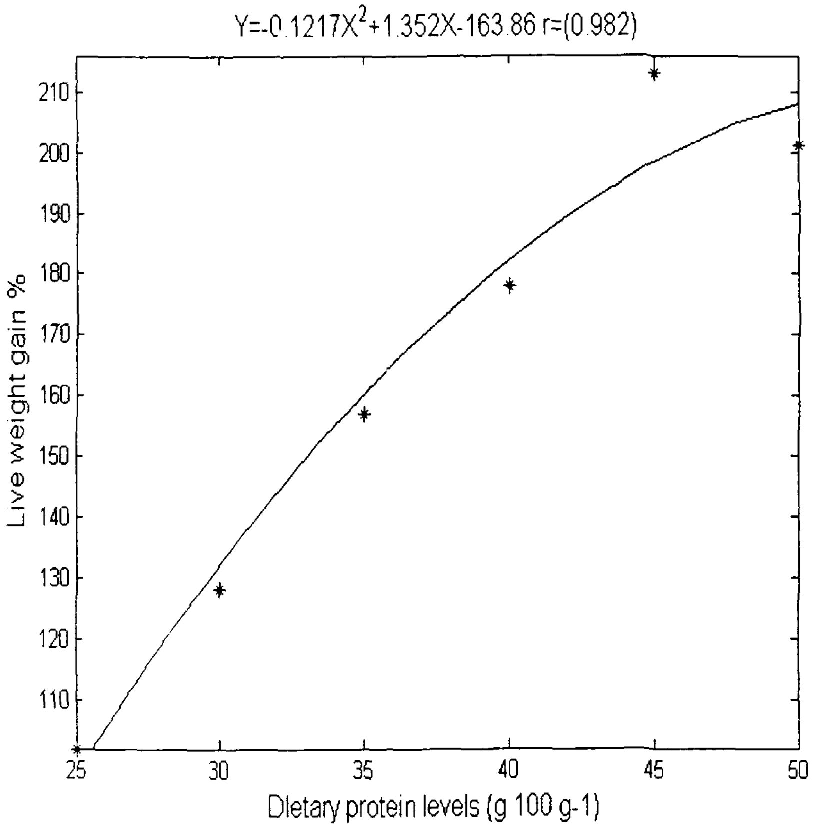
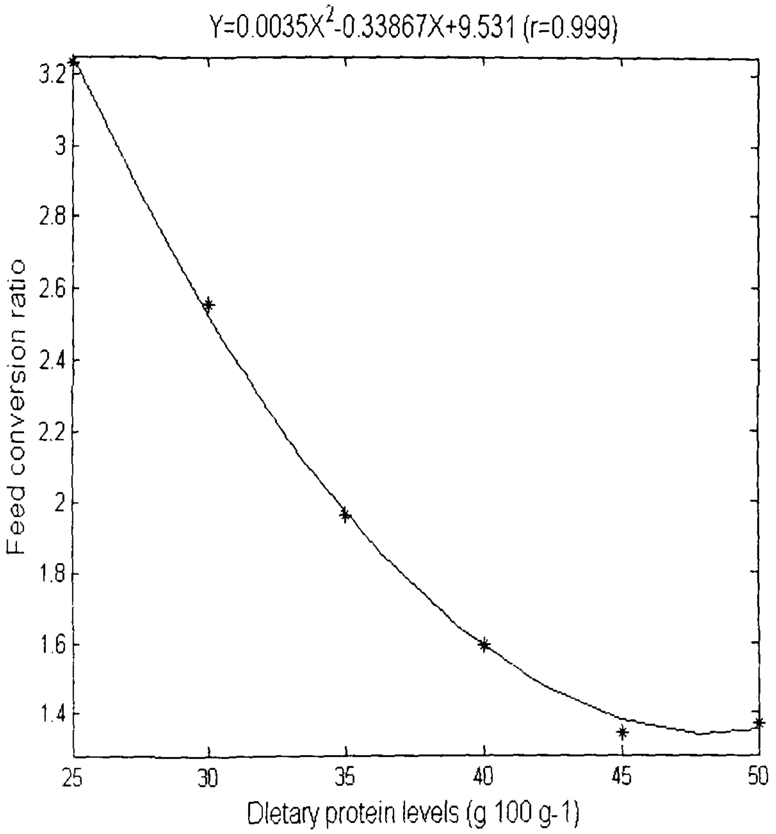
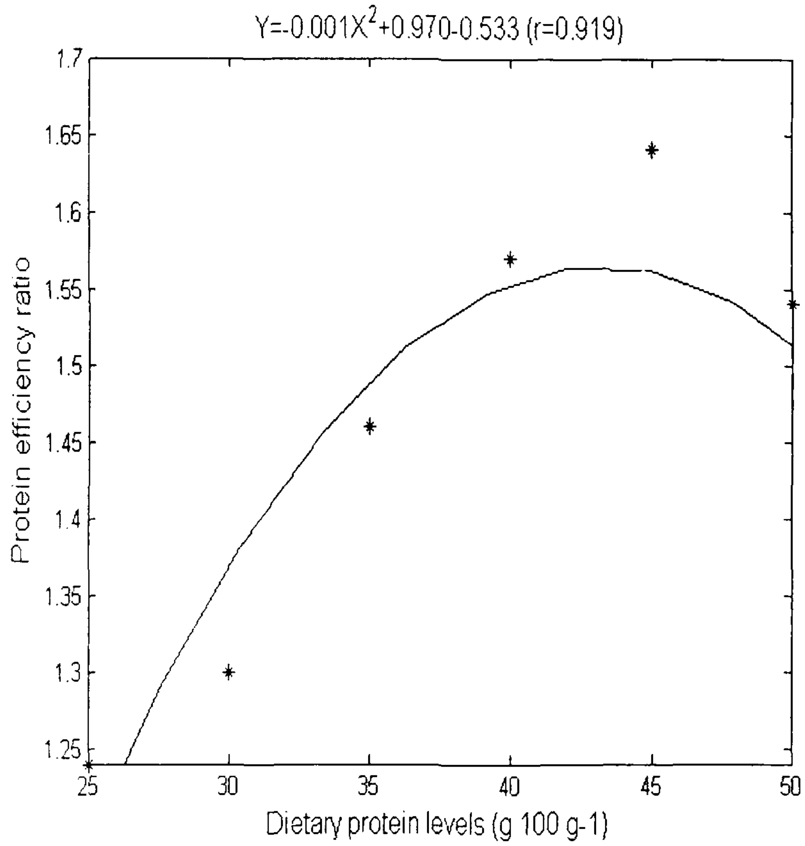

Y=-0.1217X^2+1.352X-163.86 r=(0.982)
Live weight gain %
Dietary protein levels (g 100 g-1)
110
120
130
140
150
160
170
180
190
200
210
25
30
35
40
45
50

Fig. 2 Second-degree polynomial relationship of feed conversion ratio to dietary protein levels



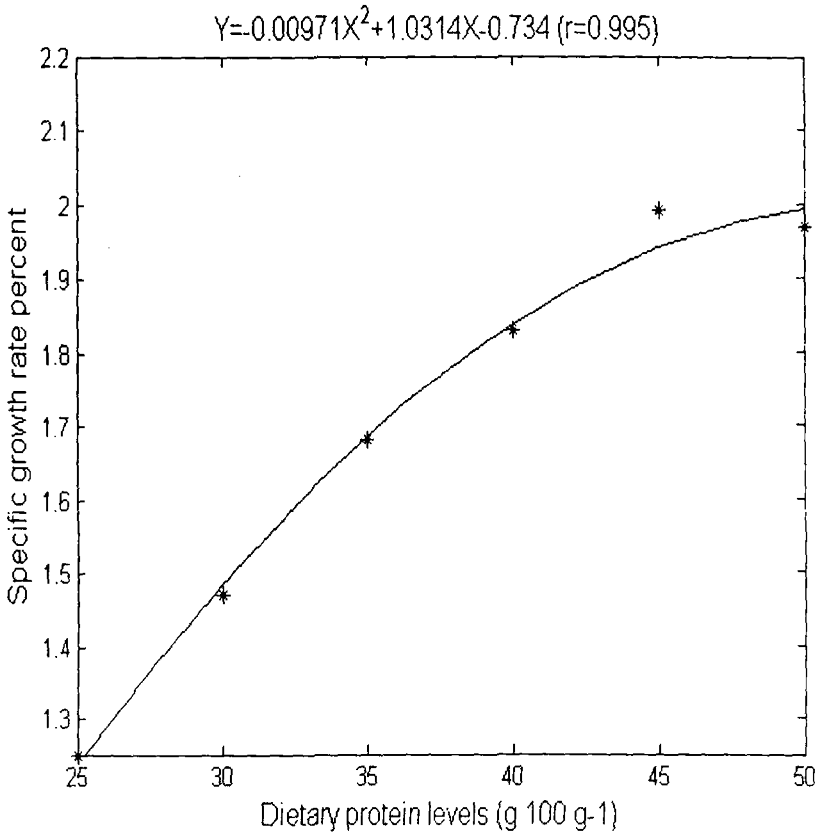
Y=0.0035X^2-0.33867X+9.531 (r=0.999)
Feed conversion ratio
Dietary protein levels (g 100 g-1)
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
25
30
35
40
45
50



Y=-0.001X^2+0.970-0.533 (r=0.919)
1.7
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
Protein efficiency ratio
25
30
35
40
45
50
Dietary protein levels (g 100 g-1)

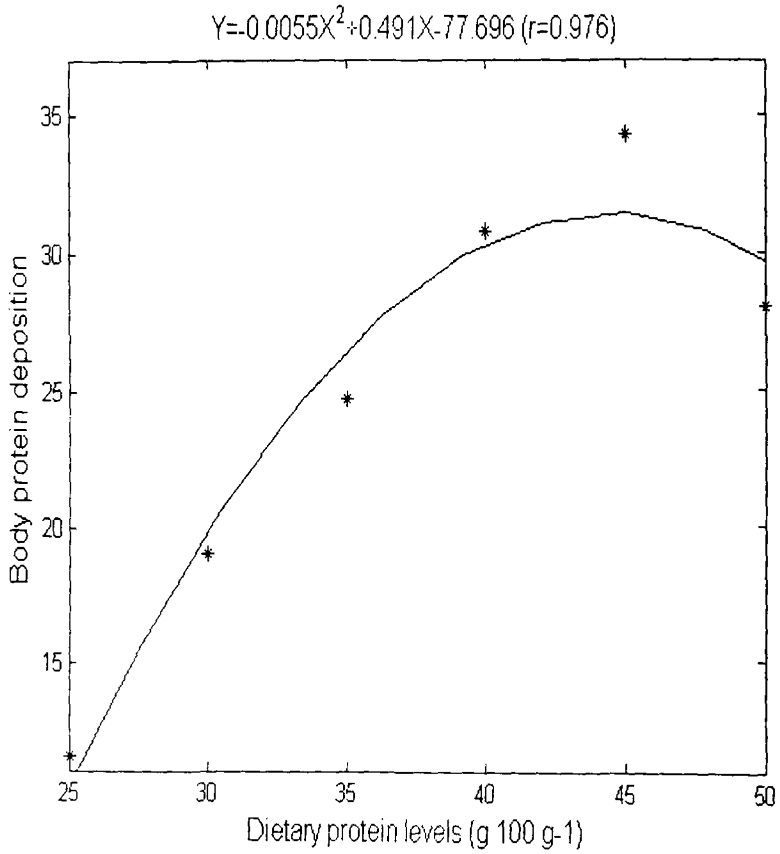
Fig. 3 Second-degree polynomial relationship of protein efficiency ratio to dietary protein levels

Fig. 4 Second-degree polynomial relationship of specific growth rate to dietary protein levels



Y=-0.00971X²+1.0314X-0.734 (r=0.995)
Specific growth rate percent
Dietary protein levels (g 100 g-1)
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
25
30
35
40
45
50

Fig. 5 Second-degree polynomial relationship of body protein deposition to dietary protein levels


Y=-0.0055X^2+0.491X-77.696 (r=0.976)
Body protein deposition
Dietary protein levels (g 100 g-1)
35
30
25
20
15
25
30
35
40
45
50

CHAPTER 2

CHAPTER 2

DIETARY PROTEIN REQUIREMENT OF YOUNG *HETEROPNEUSTES FOSSILIS* (BLOCH)

INTRODUCTION

The intensification of a fish culture has led to a dependence on artificial feeds. Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish and feed cost (Lovell 1989; Luo et al. 2004). Reducing the feeding costs could be key factor for successful development of aquaculture. Fish have high dietary protein requirement (Deng et al. 2006). The significance of qualitative and quantitative feeds is well recognized (Jauncey 1982; Mohanty & Samantary 1996; Gunasekera et al. 2000; Yang et al. 2002; Giri et al. 2003). Dietary protein requirement of a fish species is of fundamental importance in aquaculture, because dietary protein significantly influences growth, survival and yield of fish as well as economics of a farming industry by determining the feed cost which is typically the largest operational cost in aquaculture. Increase in dietary protein has often been associated with higher growth rate in many species. However, there is a protein level beyond which further growth is not supported, and may even decrease (Mohanty & Samantaray 1996; Shiau & Lan 1996; McGoogan & Gatlin 1999; Gunasekera et al. 2000; Kim & Lall 2001; Yang et al. 2002). Considerable research effort has expended to determine the quantity and quality of dietary protein necessary to achieve optimum performance of fish.

H. fossilis, the fish under study, is preferred by many fish farmers because of its ability of efficiently utilizing animal and plant origin feedstuffs and withstanding adverse environmental condition, in addition to its medicinal and good market value and potential for intensive culture. Information on the basis nutritional requirement and feeding of *H. fossilis* is needed in view of emphasis on catfish culture in the country (Tripathi & Das, 1976; Dehadrai & Thakur 1980). Protein requirements are generally higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Data on protein requirements of other cultivable fish are available (Akand et al. 1989; De Silva et al. 1989; Lochmann & Phillips 1994; Webster et al. 1995; Shiu & Lan 1996; Tibbetts et al. 2000; Islam & Tanaka 2004) but no published data is available for the protein requirement of young stage of this fish. The present work was, therefore, aimed at determining the optimum protein requirement of young *H. fossilis* for maximum growth.

Materials and methods

Preparation of experimental diets

Six casein-gelatin based isocaloric (17.37 kJ g^{-1}) diets containing graded levels of protein in the diet were formulated (Table 1). The dietary range necessary to quantify the protein requirement was adjusted on the basis of information available on other catfishes. Diets were prepared taking into account the amount of protein contributed by casein and gelatin. Diets were made isocaloric by adjusting the dextrin. In diets used for determining the protein requirement, the levels of protein were in an increment of 5 g100g^{-1} of the diet.

Method of preparation of experimental diets has been discussed under general methodology section (Page 9-10).

Feeding trial

Source of the fish, their acclimation and details of the general experimental design has already been discussed under the general methodology section (Page 10).

Young *H. fossilis* (10.03 ±0.07 cm; 10.88 g± 0.09 g) were stocked randomly in triplicate groups in 70-L circular polyvinyl troughs (water volume 55L) fitted with a continuous flow-through system at the rate of 10 fish per troughs for each dietary treatment levels. The fish were fed experimental diets in the form of moist cake to apparent satiation divided over two feeding schedules at 0900 and 1630h. No feed was offered to the fish on the day they were weighed. The feeding trials lasted for 8 weeks. Initial and weekly body weights were recorded on a top loading balance (Precisa 120A; PAG. Oerlikon, AG, Zurich, Switzerland). Troughs were siphoned off to remove faecal matter before feeding daily. Any uneaten feed was siphoned off immediately, dried in a hot air oven and reweighed to measure the amount of feed consumed. Water quality parameters were recorded daily during the feeding trial (APHA 1992). The average water temperature, dissolved oxygen, free carbon dioxide, pH and total alkalinity based on daily measurements were 27.5-28.9 °C, 67-7.1 mg L^{-1}, 5.5-10.7 mg L^{-1}, 7.5-7.8 and 65.7-80.5 mg L^{-1}, respectively.

Statistical analyses

All growth data were subjected to analysis of variance (Snedecor & Cochran 1968; Sokal & Rohlf 1981). Differences among treatment means were determined by Duncan's Multiple Range Test at a $P<0.05$ level of significance (Duncan 1955). To predict more accurate response to the dietary intake, the break-point for optimum dietary protein requirement was estimated using second-degree polynomial regression analysis ($Y=aX^2+bX+c$) as described by Zeitoun *et al* (1976). Statistical analysis was done using Matlab and SPSS

Chemical analysis

Proximate composition of casein, gelatin, experimental diets, and initial and final carcass was analyzed using standard methods (AOAC 1995) for dry matter (oven drying at 105±1 °C for 22 h), crude protein (N-Kjeldahl X 6.25 using Kjeltec Tecator™ Technology 2300, Foss, Hoeganaes, Sweden), crude fat (solvent extraction with petroleum ether B.P 40-60 °C using Socs Plus, Pelican equipments, Chennai, India, for 2-3 h.) and ash (oven incineration at 650 °C for 4-6 h). Gross energy content was determined on a Gallenkamp ballistic bomb calorimeter-CBB 330 010L (Gallenkamp, Loughbrough, U K) Six subsamples of a pooled sample of 10 fishes were analyzed for initial body composition. At the end of the experiment, 5 fishes from each replicate of dietary treatments were pooled separately and analyzed for final body composition.

Results

Growth parameters of young *H fossilis* fed diets containing graded levels of protein are presented in Table 2 Live weight gain percent, FCR, PER and SGR were sensitive to the levels of protein in the diet and improved significantly ($P<0.05$) as dietary protein level increased from 25 to 40% of the diet and at 45% dietary protein growth responses were almost constant or slightly reduced ($P>0.05$) However, a significant fall in growth and conversion efficiencies was noted at 50% protein of the diet Maximum live weight gain

(167%), best FCR (1.42), highest PER (1.65) and SGR (1.76) were obtained for the fish fed diet with 40% protein. Fish fed lower levels of protein in the diet exhibited significantly less ($P<0.05$) growth and reduced feed utilization efficiency. Poorest FCR (3.52), lowest PER (1.14), SGR (1.04) were observed for fish fed diet containing 25% protein (Diet I).

At the end of the experiment, significant differences ($P<0.05$) in whole body composition were noted among the groups (Table 3). Minimum moisture was recorded for the groups reared on 40% protein in the diet. Maximum body protein was recorded in fish fed diet containing 40% protein (Diet IV). Fish fed diets containing more than 40% protein did not show any improvement in its body protein content. Significant fall in body protein was recorded in fish fed diet containing 45% (Diet V) and 50% protein (Diet VI). Similar trend was evident for body protein deposition (BPD) values which increased significantly ($p<0.05$) with the increase in dietary protein up to 40% protein (Diet IV) and beyond which a significant fall was recorded. However, a significant and continuous increase in body fat of the fish was noted with the increase in protein content of the diet from 25% (Diet I) to 50% (Diet VI). Ash content remained unaffected throughout the treatment levels.

On subjecting the live weight gain data to quadratic regression analysis (Zeitoun *et al.* 1976), a break-point was evident at 42.75% protein of the diet. The relationship was described by the following equations:

$$Y=-0.272X^2+2.3272X-338.445 \ (r=0.975)$$

The FCR of young *H. fossilis* fed 40% protein differed significantly from the other levels of dietary protein inclusion. The FCR (Y) to dietary protein level (X) relationship was estimated by the following second-degree polynomial regression equation:

$$Y=0.005X^2-0.48978X+12.349 \ (r=0.998)$$

Based on the above equation, the estimated FCR occurred at approximately 41.3% protein of the diet.

The PER of young *H. fossilis* fed 40% protein diet differed significantly from the other levels of dietary protein inclusion. The PER (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=-0.0021X^2+0.1675X-1.793 \ (r=0.969)$$

Based on the above equation, the estimated PER occurred at a dietary protein level of approximately 40.43% of the diet.

Also, the SGR of young *H. fossilis* fed 40% protein diet differed significantly from the other levels of dietary protein inclusion. The SGR (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=0.00238X^2 +0.2007X -2.530 \ (r=0.981)$$

Based on the above equation, the estimated SGR occurred at a dietary protein level of approximately 42.16% of the diet.

Similarly, the BPD of young *H. fossilis* fed 40% protein diet differed significantly from the other levels of dietary protein inclusion. The BPD (Y) to dietary protein level (X) relationship was estimated by the second-degree polynomial regression equation:

$$Y=-0.0551X^2+0.4511X-60.25 \ (r=0.895)$$

Based on the above equation, the estimated BPD occurred at a dietary protein level of approximately 40.9% of the diet.

On the basis of the above polynomial equations, the maximum live weight gain percent, best FCR, PER, SGR and highest BPD occurred at 42.2, 41.3, 40.4, 42.2 and 40.9% protein in the diet, respectively.

Discussion

The world's demand for aquatic source of food is on the rise not only because of the growing population, but also because of a preference for healthier food (Abimorad & Carniero 2007). Feed is a major operating cost for most aquacultural operations and with the rapid expansion in aquaculture globally, fish feed research has dramatically increased in recent years. In the present study growth and conversion efficiencies increased with increasing dietary protein levels from 25 to 40% of the diet and at 45% dietary protein growth responses were almost constant or slightly reduced ($P>0.05$). However, a significant fall in growth and conversion efficiencies was noted at 50% protein of the diet indicating that 40% protein diet (Diet IV) satisfied the requirement and is considered optimum for achieving maximum growth and excellent conversion efficiency. Although feeding 45% protein diet did not show any detrimental effect neither on growth parameters nor on body composition but would be uneconomical and therefore inclusion of 40% protein in the diet for young *H fossilis* is appropriate. Similar trend was evident in body protein profile of the fish fed diets with different levels of protein. Decrease in the protein utilization beyond requirement level of dietary protein is a well-documented phenomenon (Jobling and Wandsvik 1983; Daniels and Robinson 1986; Yang et al. 2002; Tibbetts et al. 2000; Ng et. al. 2001; Kim et al. 2002; Lee et al. 2002; Deepak & Garg 2003; Yang et al. 2003; Sales et al. 2003; Kalla et al. 2004; Catacutan et al. 2001; Islam & Tanaka 2004; Luo et al. 2004; Jacinto et al. 2005, Kim & Lee 2005, Cho et al. 2005; Tibbetts et al. 2005; Sa et al. 2006). In the present study PER increased with increasing levels of protein up to 40% and slightly decreased in fish fed 45% protein diet (Diet V). However, a significant fall in PER was noted in fish fed 50% protein diets (Diet VI) indicating that 45% protein diet contains 5% extra protein which probably was not toxic to the fish. However, 10% extra protein diet (Diet VI) to the requirement might have become a burden on to the body of the fish requiring extra energy expenditure to catabolize and excrete the excess protein which resulted not only in depressing growth parameters but also lowered body protein content and deposition.

From the quadratic regression analysis of the growth and body composition data the optimum protein requirement for maximum growth of young *H. fossilis* is found to be in the range of 40.2-42.2% of the diet. The value obtained during the present study is higher than the values reported for young grey mullet, *Mugil capito* 24, (Papaparaskeva et al. 1986), Nile tilapia, *Oreochromis niloticus* 25 (El-Saidy & Gaber 2005), juvenile golden shiners, *Notemigonus crysoleucas* 29 and goldfish, *Carassius auratus* 32 (Lochmann & Philips 1994), walking catfish, *Clarias batrachus* 30 (Chaupoehuk 1987), Nile tilapia, *O. niloticus* 30 (Siddiqui et al. 1988), shingi, *Heteropneustes fossilis* 27.73-

35.43% (Akand et al. 1989), big head carp, *Aristichthys nobilis* 30 (Santiago & Reyes 1991), juvenile silver perch, *Bidynus bidynus* 31 (Yang et al. 2002), South African abalone, *Haliotis midae* 35.87 (Sales et al. 2003), lower than the requirement for Nile tilapia, *O. niloticus* 45 (El-Sayed & Teshima 1992), African catfish, *C. gariepinus* 43 (Ali & Jauncey 2005), bagrid catfish *M. nemurus* 44 (Ng et al 2001), grouper, *Epinephelus malabaricus* 44 (Shiau & Lan 1996) American eel, *Anguilla rostrata* 47 (Tibbetts et al. 2000), juvenile olive flounder, *Paralichthys olivaceus* 46.4-51.2 (Kim et al. 2002), *Salmo trutta* 57 (Arzel et al 1995) and comparable to the requirement for African catfish, *Clarias gariepinus* 40 (Degani et al. 1989), *C. batrachus* 40 (Khan & Jafri 1990), juvenile masu salmon, *Oncorhynchus masuo* 40 (Lee & Kim 2001) and Malaysian catfish, *Mystus nemurus* 42 (Khan et al. 1993) per cent protein. The differences in protein requirements among these fish species may be due to different dietary formulations, fish sizes, and differences between species or different methodology applied (Luo et al. 2004; Tibbetts et al. 2005; Sa et al. 2006).

Dietary protein requirement of young H. *fossilis* is found to be in the range of 40.2-42.2% of the diet. Data generated during the present study would be useful in developing protein balanced diets for the intensive culture of the young stage of this fish.

Summary

Dietary protein requirement of young *H. fossilis* (10.03±0.07 cm; 10.88± 0.09 g) was determined by feeding six casein-gelatin based isocaloric (17.37 kJ g^{-1}, GE) experimental diets with graded levels of protein in the diet (25, 30, 35, 40, 45 and 50% of the diet) for 8 weeks in triplicate groups. Live weight gain percent (167%), PER (1.65) SGR (1.76) and BPD (37.46) were significantly higher in fish fed diet containing 40% protein in the diet. Best FCR (1.46) was also recorded at this level. Second-degree polynomial regression analysis of the live weight gain, FCR, PER, SGR and BPD data indicated the dietary protein requirement of young *H. fossilis* to be at 42.75, 41.3, 40.4, 42.2, and 40.9% of the protein, respectively. Body composition data also supported the above requirement level. Based on the quadratic regression analysis data dietary protein requirement of young *H. fossilis* is estimated to be in the range of 40.2-42.7% of the diet.

Table 1 Composition of experimental diets used for quantifying the dietary protein requirement of young *H. fossilis.*

	Diets					
Ingredients (g 100g^{-1}, dry diet)	**I**	**II**	**III**	**IV**	**V**	**VI**
Casein[1]	25	30	35	40	45	50
Gelatin[2]	6.25	7.50	8.75	10.0	11.25	12.5
Dextrin	49.74	40.96	32.18	23.39	14.61	5.83
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[4]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[4,5]	3.0	3.0	3.0	3.0	3.0	3.0
α- Cellulose	-	2.53	5.07	7.6	10.14	12.67
Carboxymethyl cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Total	99.994	99.993	100	99.996	100	100
Calculated crude Protein	25.0	30.0	35.0	40.0	45.0	50.0
Analyzed crude Protein	24.93	29.19	34.45	39.65	44.15	49.03
Gross energy[6] (kJ g^{-1}, dry diet)	17.34	17.34	17.34	17.34	17.34	17.34

[1]Crude Protein (80%), Loba Chemie, India; [2]Crude Protein (97%), Loba Chemie, India.; [3]Loba Chemie, India.; [4]Halver (2002); [4,5]1g Vitamin mix +2g α-cellulose; [6]Calculated on the basis of fuel values 23.08, 20.199, 16.02 and 37.64 kJ g^{-1} for casein, gelatin, dextrin, and fat, respectively, as estimated on Gallenkamp ballistic bomb calorimeter.

Table 2 Growth and conversion efficiency of young *H. fossilis*

	Dietary protein levels					
	25	**30**	**35**	**40**	**45**	**50**
Average initial weight (g)[1]	10.03 ± 0.01^{a}	10.08 ± 0.01^{a}	10.04 ± 0.01^{a}	10.05 ± 0.01	10.05 ± 0.02^{a}	10.14 ± 0.07^{a}
Average final weight (g)[1]	17.95 ± 0.06^{d}	20.85 ± 0.05^{b}	23.65 ± 0.02^{b}	26.87 ± 0.06^{a}	26.18 ± 0.07^{a}	23.92 ± 0.02^{b}
Live weight gain [1,2]	79.03 ± 6.3^{f}	106.9 ± 10.9^{e}	135.5 ± 9^{d}	167.4 ± 6^{a}	160.4 ± 7^{b}	140.6 ± 8^{c}
Food conversion ratio[1,2,3]	3.52 ± 0.02^{a}	2.55 ± 0.04^{b}	1.89 ± 0.01^{c}	1.42 ± 0.01^{d}	1.43 ± 0.02^{d}	1.45 ± 0.04^{e}
Protein efficiency ratio[1,2,4]	1.14 ± 0.01^{f}	1.30 ± 0.03^{e}	1.51 ± 0.09^{c}	1.65 ± 0.24^{a}	1.56 ± 0.03^{b}	1.38 ± 0.03^{d}
Specific growth rate (%)[1,2,5]	1.04 ± 0.08^{e}	1.29 ± 0.01^{d}	1.52 ± 0.09^{c}	1.76 ± 0.05^{a}	1.71 ± 0.02^{a}	1.53 ± 0.02^{b}
Percentage survival	100	100	100	100	100	100

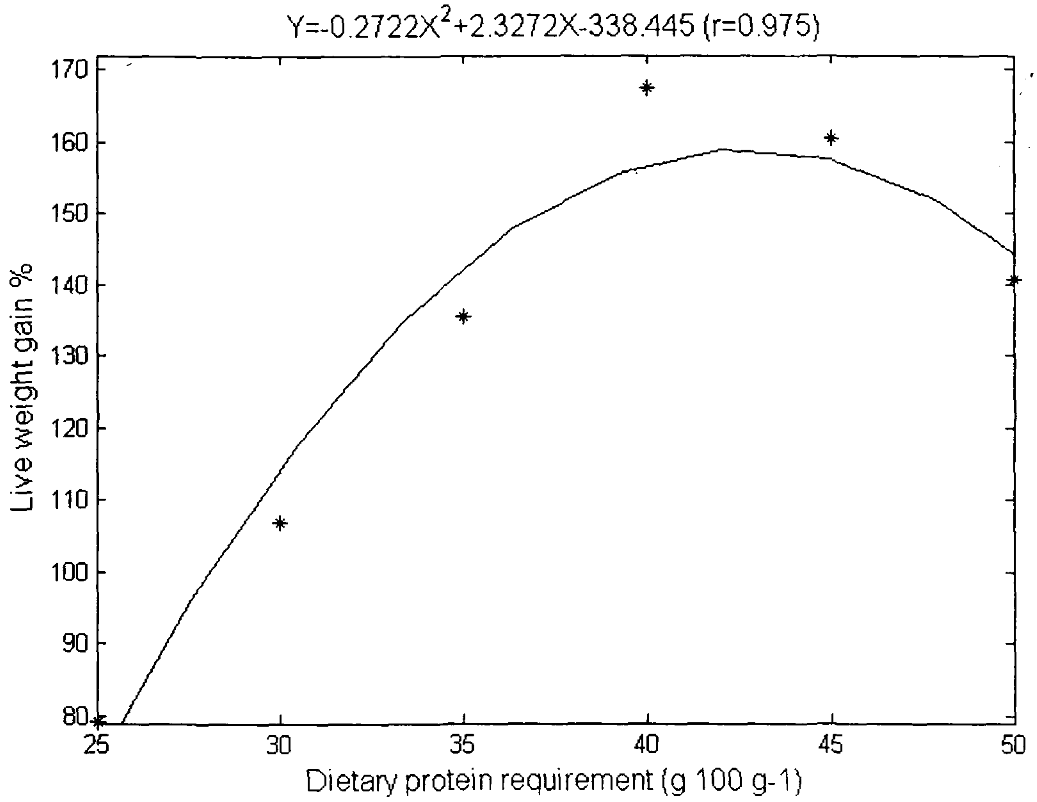
[1] Mean values of 3 replicates ± SEM; [2] Mean values sharing the same superscripts are insignificantly different ($P>0.05$).
[2]Live weight gain= Final body weight-Initial body weight/Initial body weight x 100
[3]FCR= Dry feed fed/Weight gain
[4]PER= Wet weight gain/Protein fed
[5]SGR%= ln Final body weight-ln Initial body weight/Number of days x 100

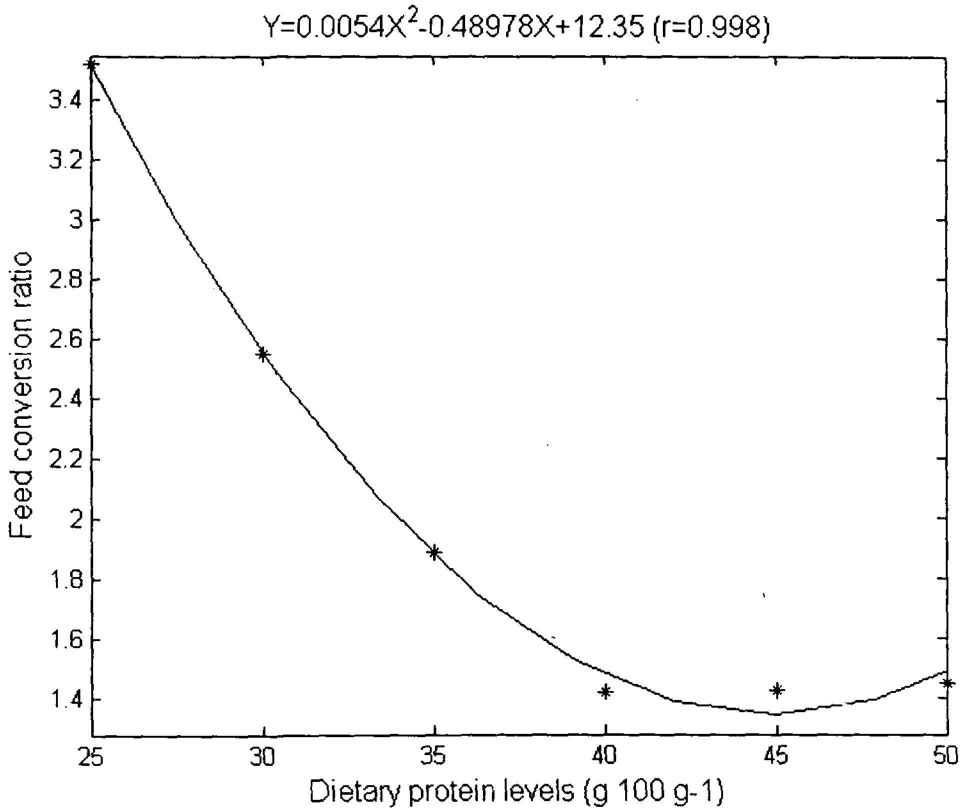
Table 3 Body composition of young *H. fossilis*

		Dietary protein levels					
		25	**30**	**35**	**40**	**45**	**50**
Moisture	80.98± 0.22	79.01 ± 0.11^{a}	78.55 ± 0.10^{b}	77.67 ± 0.15^{c}	76.07 ± 0.05^{d}	76.33 ± 0.30^{d}	76.98 ± 0.12^{e}
Protein	11.92± 0.22	12.11 ± 0.33^{d}	12.95 ± 0.24^{d}	14.69 ± 0.30^{c}	15.76 ± 0.15^{a}	15.12 ± 0.60^{a}	15.01 ± 0.60^{b}
Fat	5.54± 0.05	6.01 ± 0.10^{f}	6.99 ± 0.10^{e}	7.23 ± 0.03^{d}	7.98 ± 0.10^{c}	8.23 ± 0.15^{b}	8.91 ± 0.16^{a}
Ash	3.63±0.05	4.23 ± 0.04^{a}	3.87 ± 0.40^{ab}	3.92 ± 0.10^{ab}	3.51 ± 0.03^{b}	4.09 ± 0.40^{ab}	3.55 ± 0.04^{b}
Body protein deposition	-	18.21 ± 0.07^{e}	22.17 ± 0.04^{d}	27.99 ± 0.07^{c}	37.46 ± 0.06^{a}	29.65 ± 0.06^{b}	20.32 ± 0.27^{d}

[1] Mean values of 3 replicates ± SEM; [2] Mean values sharing the same superscripts are insignificantly different ($P>0.05$).

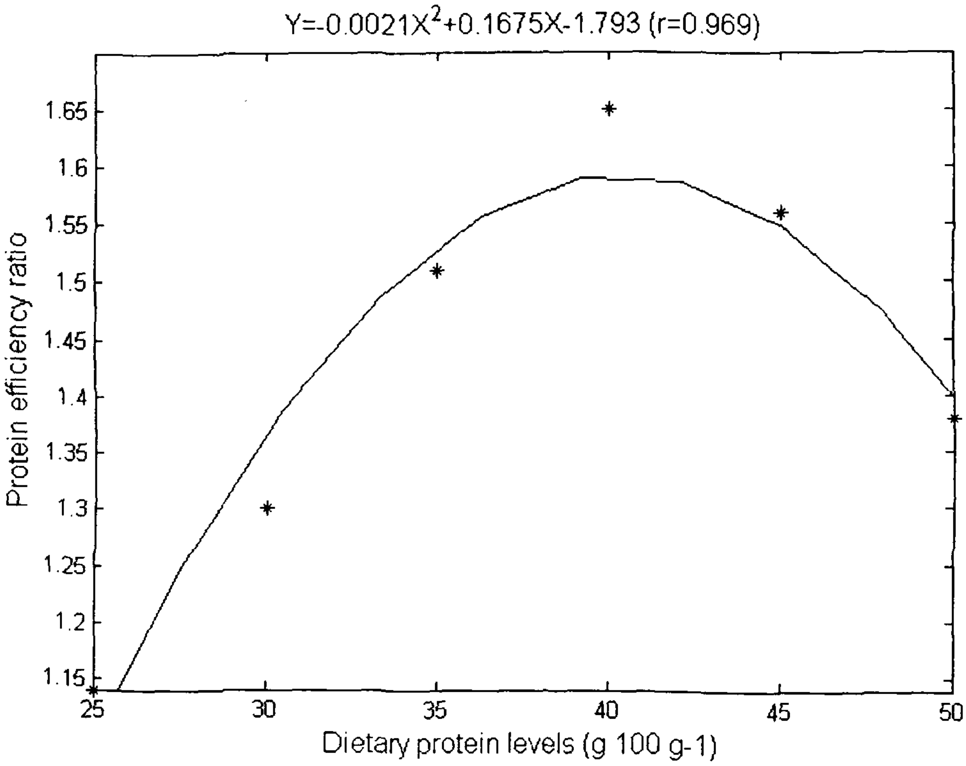
Fig. 1 Second-degree polynomial relationship of live weight gain per cent to dietary protein levels


Y=-0.2722X^2+2.3272X-338.445 (r=0.975)
Live weight gain %
Dietary protein requirement (g 100 g-1)
170
160
150
140
130
120
110
100
90
80
25
30
35
40
45
50



Y=0.0054X²-0.48978X+12.35 (r=0.998)
Feed conversion ratio
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
25
30
35
40
45
50
Dietary protein levels (g 100 g-1)

Fig.3 Second-degree polynomial relationship of protein efficiency ratio to dietary protein levels



Y=-0.0021X2+0.1675X-1.793 (r=0.969)
Protein efficiency ratio
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
1.2
1.15
25
30
35
40
45
50
Dietary protein levels (g 100 g-1)

Fig. 4 Second-degree polynomial relationship of specific growth rate per cent to dietary protein levels

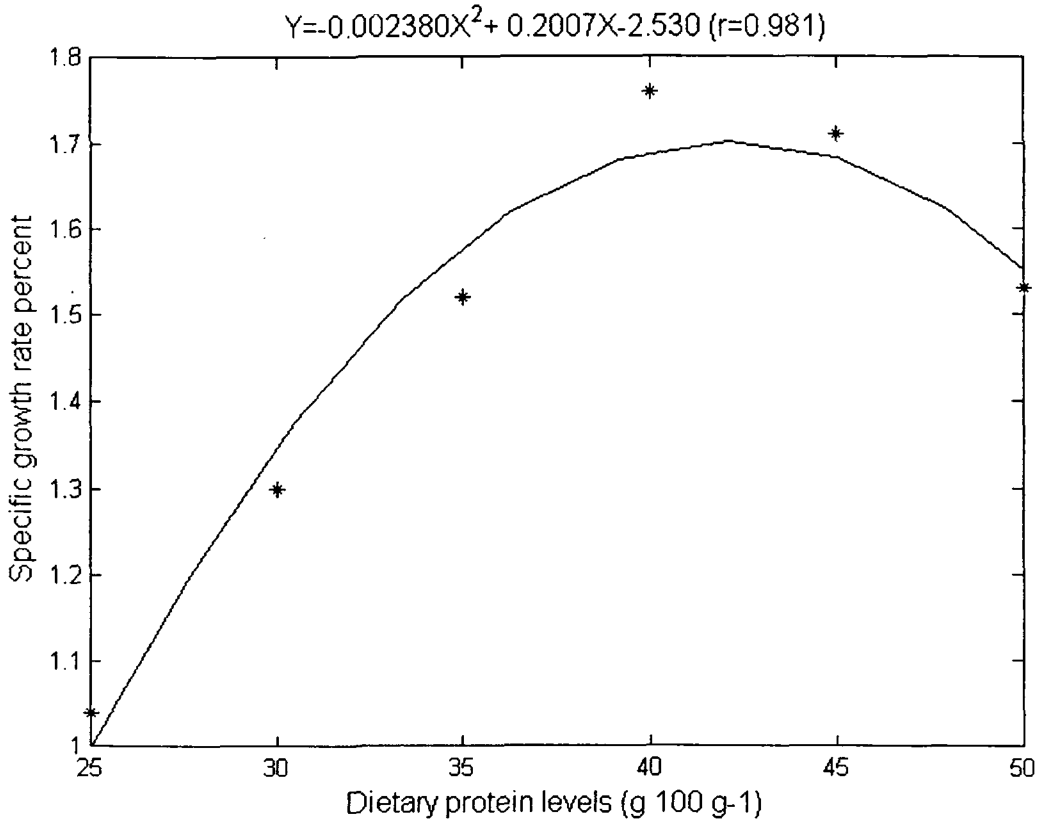
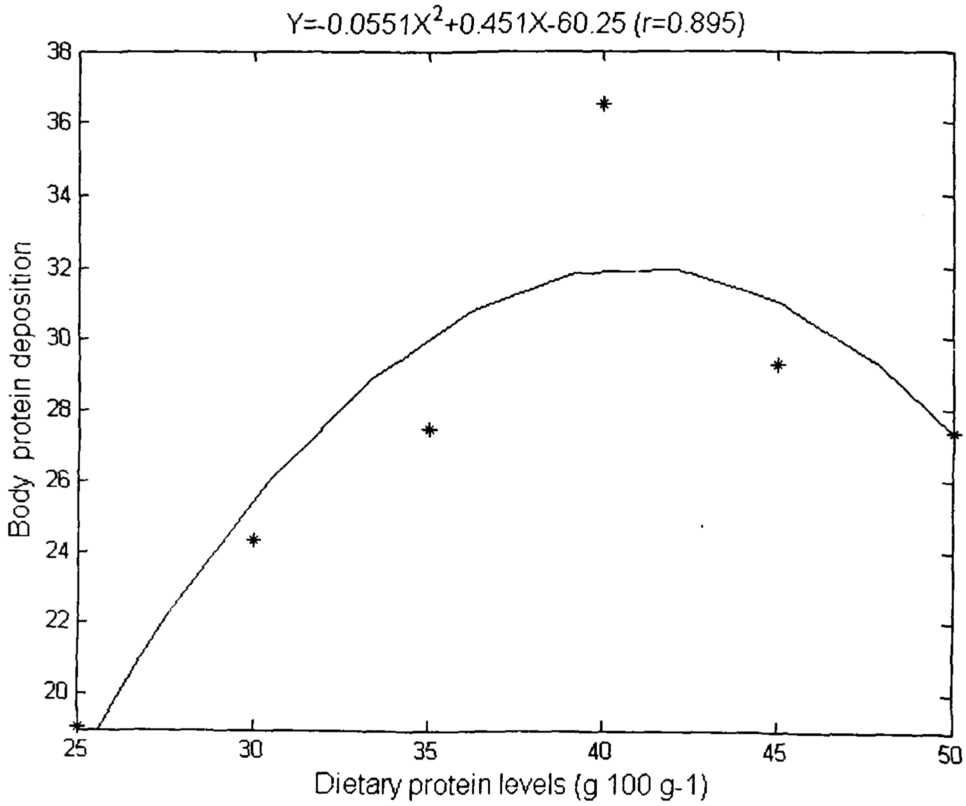

Y=-0.002380X^2+ 0.2007X-2.530 (r=0.981)
Specific growth rate percent
Dietary protein levels (g 100 g-1)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
25
30
35
40
45
50

Fig.5 Second-degree polynomial relationship of body protein deposition to dietary protein levels


Y=-0.0551X²+0.451X-60.25 (r=0.895)
38
36
34
32
30
28
26
24
22
20
Body protein deposition
25
30
35
40
45
50
Dietary protein levels (g 100 g-1)

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